

Tag No. \_\_\_\_\_

purpose: continuation of pg 124 - 125

amplified linearized puc / xmn 1 using 2 different new sets of primers

36

37

38

39

Tried with Tag and Tag + D.V.

|       |    |                       |         |             |
|-------|----|-----------------------|---------|-------------|
| thiol | Mg | 1.5, 2.0, 2.5, 3.0 mM | cycling | 94° 30" 1   |
|       |    |                       |         | (94° 30" 30 |
|       |    |                       |         | 68° 1' 30   |

200  $\mu$ M dNTP1.4  $\mu$ M primer

product = 1.275 bp.

1 U of enzyme - Tag

25  $\mu$ g template

|         |     |         |     |   |     |            |   |     |
|---------|-----|---------|-----|---|-----|------------|---|-----|
| reagent | 10x | of each | Tag | / | # 3 | Tag + D.V. | / | # 3 |
|         |     |         | "   | / | # 2 | "          | / | # 2 |

1/20 338 230 ml

10x buffer 50

dNTP 10

Mg -

primer 1 20

2 20

template 10

enzyme 2

- 10  $\mu$ l Tag + D.V.

450

45  $\mu$ l / 1.5 added 5  $\mu$ l of Mg div. cov.

To Page No. \_\_\_\_\_

Used &amp; Understood by me,

Date

12/9/94

Invented by

Recorded by

A. Sitarman

Date

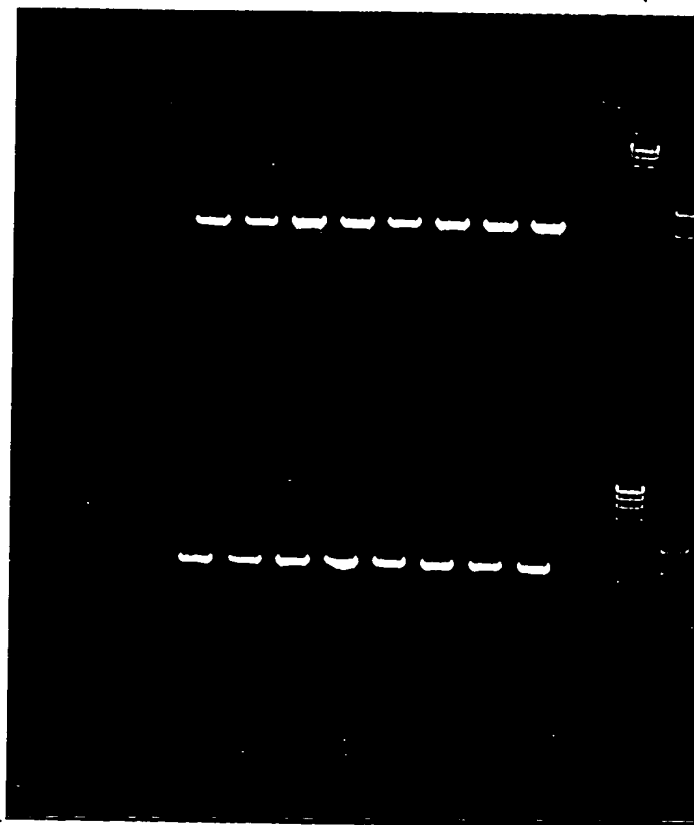
12/9/94

From Page No. \_\_\_\_\_

Tag 0 1.5 2 2.5 3 mm Mg

\*3

\*2



Tag + DV

0 1.5 2 2.5 3 mm

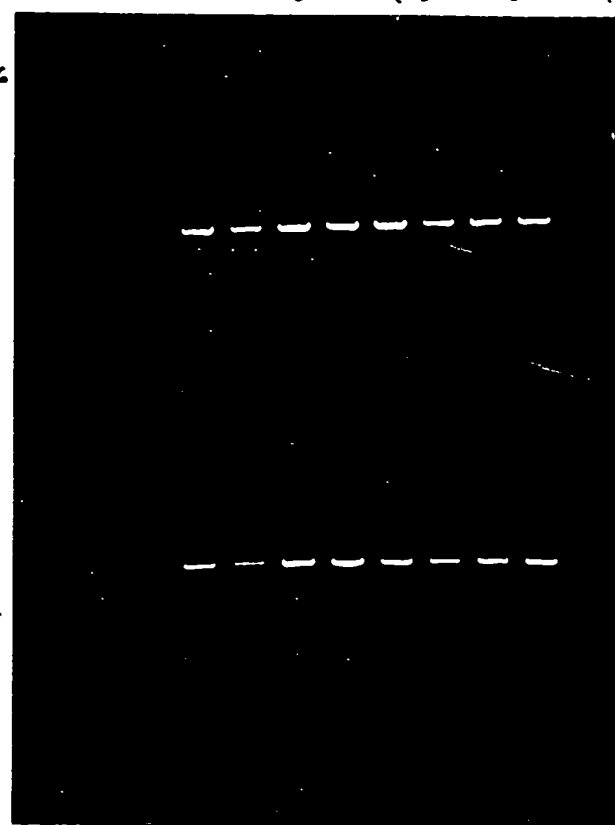
2936

v

37

\*3

\*2



2936 x 37

✓ 1275 bp product.

- Both primers set work with Tag as well as Tag + DV  
 a bit of mispriming still - has to be gel purified  
 - great range of Mg tolerance.

pooled (1) Tag 1.5 mm Rx Separately } with \*3  
 (2) Tag 2.0 } set of  
 (3) Tag + DV 1.5 } primer  
 (4) Tag + DV 2.0 } and phenol ex  
 ethanol p/

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Witnessed &amp; Understood by me,

Date

12/19/94

Invented by

Recorded by

A. Sitarman

Date

12/07/94

Tag N. \_\_\_\_\_

loaded R<sub>x</sub> from two tubes (duplicate of same) together in 30  $\mu$ l +  
made up the volume to 100  $\mu$ l 30  $\mu$ l

added equal amount of phenol: chloroform: 2x amylalcohol

removed the aqueous phase after a spin of 5'

phenol extracted again.

added 0.5 volume of 7M ammonium acetate and 2 vol  
of ethanol, added also a  $\mu$ l of dextran T 500

left at  $-20^{\circ}$ , 1.5 hr

spin down, remove ethanol, washed the pellet with  
70% ethanol, spin down, remove the sup.

spin again to remove the residual ethanol

pellet visible, vacuum dried 5'

resuspended in 17  $\mu$ l of <sup>20</sup> TB - removed 2  $\mu$ l for gel

for 15  $\mu$ l added

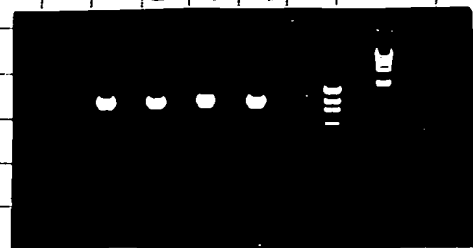
10.5 " H<sub>2</sub>O

3.0 " 10x buffer

1.0 " Afl III (7U/x)

0.5 " Afl II (24U/x)

④ ③ ③ ④



30  $\mu$ l incubated at  $37^{\circ}$ , 2 hr.

phenol extracted product seems to be around ~ 150 - 200 ng / x 2

~ 75 ng / x

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is d &amp; Understood by me,

Date

12/1/84

Invented by

Recorded by

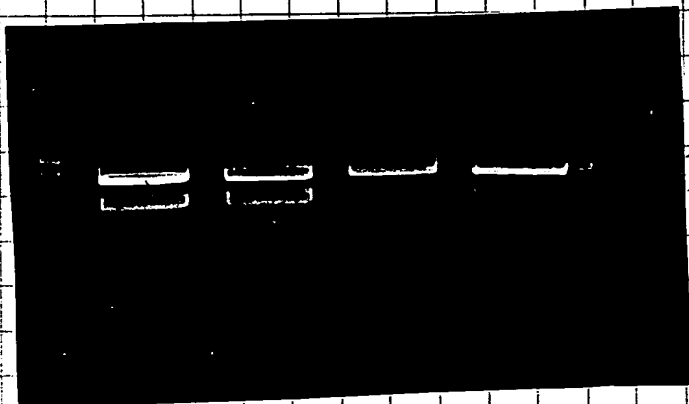
K. Sitarman

Date

12/1/84

Form Page No. \_\_\_\_\_

- 15  $\mu$ l of left over phenol chloroform extracted & ethanol pptd. insert & was cut with Pst I and Pst 3 in NEB buffer 4 for 2 hrs at 37°
- Run on 1% gel and transferred to DAE paper and eluted the fragment in high salt buffer, over the 1M NaCl, 0.1M Tris pH 8.0, 5mM EDTA
- spun down the elution buffer, added 50  $\mu$ l more & centrifuged, preclt the elution, ethanol pptd in  $\sim$  150  $\mu$ l  $\rightarrow$  500  $\mu$ l in the presence of 1  $\mu$ l of deprotein T-400.
- left at 70°, 2 1/2 hrs, resuspended in 15  $\mu$ l of 95% ethanol wash, in 70°.



$$\text{loaded} \sim 75 \text{ ng} \times 15 \mu\text{l} = 1125 \text{ ng} (1275 \text{ bp})$$

$$= 772 \text{ ng} (875 \text{ bp})$$

$$\sim 50\% \text{ recovery} = \sim 386 \text{ ng} / 15 \mu\text{l}$$

$$= \sim 25 \text{ ng} / \lambda$$

To Page

Witnessed &amp; Understood by m ,

Date

12/19/94

Invented by

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K. Stamen

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12/12/94